Universität Regensburg

FAKULTÄT
CHEMIE UND PHARMAZIE
Institut für Physikalische und
Theoretische Chemie

Universität Regensburg · D-93040 Regensburg

Prof. Dr. Dominik Horinek
Telefon +49 941 943-4745
Telefax +49 941 943-4532
Sekretariat:
Telefon +49 941 943-4045/4296
Universitätsstraße 31
D-93053 Regensburg

Dominik.Horinek@chemie.uni-regensburg.de
www.uni-regensburg.de

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Evaluation Report of the thesis "Ion Specific Hofmeister Effects on Peptides and Proteins" submitted by Ing. Jana Hladilkova as Ph. D. thesis at the Univerzita Karlova V Praze

Jana Hladilkova's Ph. D. thesis deals with different aspects of ion specificity in the context of protein and peptide biochemical science. These ion specific effects are investigated theoretically based on classical molecular dynamics simulations.

The work starts with an introduction to ion specificity beginning with Hofmeister's famous work. The Hofmeister ion series is presented along with the early concept of structure breaking and structure making, which does not rely on ion-protein. The newer idea that ion-protein interactions are most important and their separation into backbone and side chain interactions are introduced.

In the chapter "Synergy of Theory and Experiment" an overview of the theoretical concepts is given. Spatial distribution functions, radial distribution functions, and proximal distribution functions are introduced. Preferential binding coefficients are introduced in their relation to Kirkwood-Buff integrals and in relation to particle numbers of solvent and ions in the vicinity of the respective group. Salting in and salting out constants are introduced, and a phenomenological description of ion effects on LCST of polymers and of molecular spectroscopy is given. Finally, ion effects on electrophoretic experiments are introduced.

After the introduction of the theoretical concepts, individual projects are described in separate chapters. The first chapter deals with ion effects on different proteins. Simulations of the enzyme BHMT are described. In this system, potassium shows the strongest ion effect. The highest probability density of cations was found close to the Asp26 and Glu159 residues. The simulations were used to predict a binding site of potassium, which was later experimentally shown to be correct. Another protein studied was the LinB microbial enzyme. The monovalent alkali ions follow a Hofmeister series, the larger the cation is, the lower its affinity for the protein is. The most relevant region of this protein for cation binding is a 'tunnel mouth'. Further insight is gained by point mutations at this location.

In the next chapter follows the discussion of a more elementary study of ion effects that looks at ion-peptide interactions in model systems: elastin-like polypeptides, which have a repetitive structure with VPGVG units. The experimental observations

that NaSCN unlike other studied salts induces a nonlinear change of the LCST and of the glycine proton shifts is explained by an affinity of the SCN ion to the NH and CH2 groups of the backbone, but is repelled by the valine side chain. The next systems studied are capped, uncapped and semi-capped triglycine peptides. A remarkable feature of this work is that a longstanding experimental result needed new interpretation as the presumed data for the capped peptide matches the results for the half-capped peptide, but not the data for the fully capped tripetide. Ion affinities for this backbone model vary significantly from strongly hydrated ions showing no affinity to weakly hydrated ions that show a strong affinity.

In the last project ion effects on the behavior of the simple backbone model N-methyl acetamide and on neutral "EOF" markers in electrophoresis experiments are studied. The example of thiourea is chosen as EOF marker. The preferential binding coefficients are determined from simulations, and from these the influence on the electrophoretic mobility is determined. The simulations predict that formally neutral compounds can show some electrophoretic mobility due to preferential ion adsorption.

The thesis ends with a conclusion, a collection of references, and seven original papers that Jana Hladilkova has coauthored.

The work presented in this thesis is a nice and rigorous application of modern molecular simulations to biological questions related to ion specificity. Crucial insight is only possibly through a combination of simulations, which reveal a detailed view of structure and dynamics on the molecular level, and experiments. The text is written in a clear scientific style, the references are appropriate, and the level of English is excellent. I can wholeheartedly confirm that this thesis meets all requirements for acceptance as a Ph. D. thesis.

I would like to address three points concerning the thesis for questions:

1) Kirkwood-Buff integrals and the preferential binding coefficient: The relation given in Eq. 3 on page 7 of the thesis is not the most original definition of the preferential binding parameter/coefficient. In fact, different 'preferential binding coefficients' have been defined in the literature. What is the thermodynamic origin of the preferential binding coefficient as it is defined in chapter 2.1.3?

2) In chapter 4.3 the half-capped tripeptide, in which only the N-terminal side is capped. What would be expected for a half-capped peptide, in which only is the C-terminal side is capped?

3) Chapters 2.3 and 4.4 describe the calculation of electrophoretic mobilities by calculating the radial distribution functions of the ions around the solute and using Altenberger's and Friedman's theory. How does this approach compare to a direct calculation of the molecule's mobility by a non-equilibrium MD simulation?

Sincerely